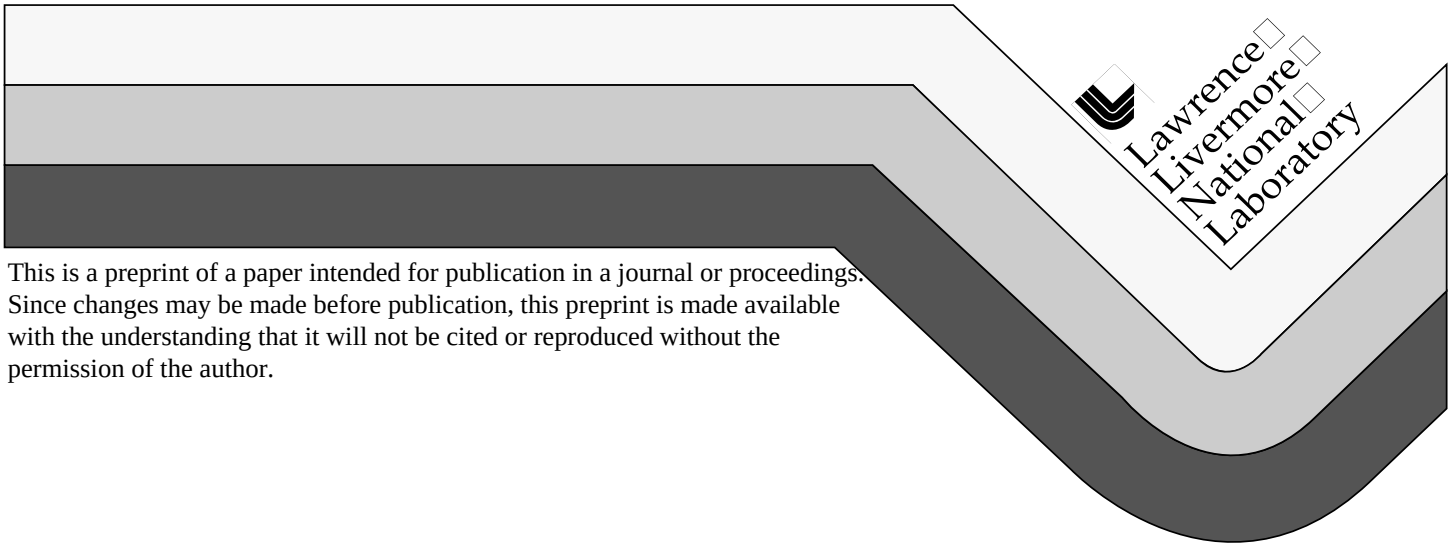
UCRL-JC-131039
Preprint

NUTRIENT LIMITATION AND MICROBIALLY MEDIATED CHEMISTRY: STUDIES USING TUFF INOCULUM OBTAINED FROM THE EXPLORATORY STUDIES FACILITY, YUCCA MOUNTAIN

A. Meike, C.-I. Chen, Y.-J. Chuu, A Sawvel, and D. Ringelberg

This paper was prepared for submittal to
Scientific Basis for Nuclear Waste Management
Materials Research Society Symposium
Boston, Massachusetts, November 30–December 4, 1998

October 30, 1998



NUTRIENT LIMITATION AND MICROBIALLY MEDIATED CHEMISTRY: STUDIES USING TUFF INOCULUM OBTAINED FROM THE EXPLORATORY STUDIES FACILITY, YUCCA MOUNTAIN

A. MEIKE*, C.-I. CHEN*, Y.-J. CHUU*, A. SAWVEL*, D. RINGELBERG**
*Lawrence Livermore National Laboratory, Livermore, California 94550
**Waterways Experiment Station, U.S. Army Corps of Engineers, Vicksburg, Mississippi 39180

ABSTRACT

Flow-through bioreactors are used to investigate the relationship between the supply (and limitation) of major nutrients required by microorganisms (C, N, P, S) and effluent chemistry to obtain data that can be useful to develop models of microbially mediated aqueous chemistry. The bioreactors were inoculated with crushed tuff from Yucca Mountain. Six of the 14 bioreactor experiments currently in operation have shown growth, which occurred in as few as 5 days and as much as a few months after initiation of the experiment. All of the bioreactors exhibiting growth contained glucose as a carbon source, but other nutritional components varied.

Chemical signatures of each bioreactor were compared to each other and selected results were compared to computer simulations of the equivalent abiotic chemical reactions. At 21°C, the richest medium formulation produced a microbial community that lowered the effluent pH from 6.4 to as low as 3.9. The same medium formulation at 50°C produced no significant change in pH but caused a significant increase in Cl after a period of 200 days. Variations in concentrations of other elements, some of which appear to be periodic (Ca, Mg, etc.) also occur. Bioreactors fed with low C, N, P, S media showed growth, but had stabilized at lower cell densities. The room temperature bioreactor in this group exhibited a phospholipid fatty acid (PLFA) signature of sulfur- or iron-reducing bacteria, which produced a significant chemical signature in the effluent from that bioreactor. Growth had not been observed yet in the alkaline bioreactors, even in those containing glucose.

The value of combining detailed chemical and community (e.g., ester-linked PLFA) analyses, long-duration experiments, and abiotic chemical models to distinguish chemical patterns is evident. Although all of the bioreactors contain the same initial microorganisms and mineral constituents, PLFA analysis demonstrates that both input chemistry and temperature determine the character of the long-term population of microorganisms. Where microbial growth occurs, that community can impact the chemistry of water significantly. These principles are well known, but we note their relevance to modeling microbially mediated chemistry. We recognize, in addition to microbial growth, three categories of chemical effects, each of which will require a different approach and constitutive equation(s): 1) unidirectional bacterial modification of the chemistry (i.e., pH) that is directly related to the dominance of particular species, 2) secondary impact of direct microbial modifications (i.e., increased dissolution of solids as a result of reduced pH), and 3) cyclical effects that may be attributed to internal regulation (e.g., osmoregulation or internal pH regulation) or evolution of the microbial community in the bioreactor.

INTRODUCTION

Chemists of natural systems are becoming increasingly aware of the role microorganisms may play in aqueous chemistry. The extent of impact that microorganisms can have is affected by the water:solid surface ratio and by the activity of the microorganisms. Presently the ability to predict aqueous chemistry is far more developed in the area of abiotic chemistry than it is in microbially mediated chemistry. Our goal has been to develop an understanding of the impact of microbes on aqueous chemistry with specific application to the potential repository at Yucca Mountain, Nevada. At present, the indigenous population of microorganisms (see, e.g., [1]) is not very active. However, nutrients and nonindigenous microbes that may be introduced as construction and auxiliary materials, in addition to the expected thermal pulse and concomitant changes in relative humidity, could significantly alter the activity of microorganisms and the chemical consequences of that activity. We plan to obtain data and identify the strategies that will allow us to integrate the effects of microbially mediated chemistry into existing computer codes that are intended to simulate aqueous chemistry.

The media for the experiments were formulated to represent limitations of a modified geological environment that is not presently nutritious for microbes but that may be altered by the thermal pulse and construction materials. Given that the chemical nature of the construction materials, which in this environment could contribute significantly to the nutrient supply, can be controlled, we investigated the relationship between the supply (and restriction) of the major nutrients required by microorganisms (C, N, P, S) and the resultant aqueous chemistry. Because one of the most significant construction materials in the high-level waste repository may be concrete, and because the greatest

impact of concrete on aqueous chemistry, especially before the thermal pulse, could be the production of an alkaline pH (as much as 12 or higher, depending on the formulation), we have included medium formulations that are quite alkaline to represent such situations in the potential repository.

EXPERIMENTAL STUDIES

Each flow-through bioreactor consists of a reservoir, a reaction chamber, and a sampling port. These are contained by sterile methods and connected by sterile tubing. Polyethersulfone filters (0.2 μm) are located at the exit port of the reaction chambers and retain all cell mass in the bioreactor. Thus, these flow-through bioreactors contrast with the chemostats of Matin [2] in which cell mass is required to escape. Flow to and from the reaction chamber is governed by a peristaltic pump having a set rate of 100 ml/day. In reality, because of progressive accumulation of debris, the nominal rate varies between 50 and 100 ml/day with a volume in the reactor remaining at roughly 400 ml. Two bioreactors are fed with each medium formulation in controlled temperature environments: one at 21°C and the other one at 50°C. Medium is supplied to each reservoir periodically to ensure a continual supply to the reactors. Each reaction chamber contains an inoculum of 100 g crushed tuff. Tubing and filters are changed periodically as the flow rate is impeded. The change in flow rate varies depending on the formulation. Effluent is sampled periodically for analysis.

Topopah Spring tuff was collected from the Exploratory Studies Facility (ESF) at Yucca Mountain, Nevada, by sterile methods and crushed and sieved aseptically to a mesh of 1.68 mm < x <1.83 mm. The crushed tuff contained indigenous bacteria from the Yucca Mountain potential repository and species introduced during the construction of the ESF.

Medium formulations were developed to represent all combinations and permutations of low, medium, and elevated (relative to the present environment) C, N, P, and S. The maximum concentrations of each element of the formulation are within the bounds of that which might seem reasonable for the potential repository environment. Our study is somewhat unique compared to most microbial studies because we expect and desire no growth under certain conditions. An examination of the standard media for microbial growth indicated that most were too nutritious for our purpose and that inclusion of minor nutrients (e.g., K, Mg) could be significant. Given that the natural groundwater contains these elements and that during the thermal pulse, water will be concentrated and saturated in various minerals that precipitate and finally may form evaporites, we expect these minor nutrients to be available. The precipitated minerals can contribute to an increase in the ionic strength of fluids migrating into the repository after the thermal pulse. Reproducing such a complex group of groundwater compositions is not important to this study. However, it is important to derive guidelines for a maximum limit to the minor constituents in our formulations from the elements contained in one of these concentrated groundwaters. We arbitrarily set this limit at 100 times the concentration of elements in J-13 well water (see e.g., [3]). The 100× values do not represent an actual aqueous composition, of course, because the actual solution would be supersaturated with respect to many minerals. The maximum concentrations of our major nutrients C, N, P, and S are constrained by the M9 [4] formulation, a basic nutrient formulation for culturing bacteria. These concentrations (or greater), at times above the 100× J-13 values, are possible if certain materials are introduced to the proposed repository. Minimum concentrations of these major nutrients could be as high as that allowed by the hypothetical 100× J-13 well water. Minor modifications necessary to balance the charge were conducted within these constraints. Note that the formulations included concentrations of Si that might be expected in the modified groundwater. Table I describes the compositions of the media supplying bioreactor experiments that are presently in progress

Growth of cells in the various bioreactors was determined by measuring the absorbance of the culture at 660 nm wavelength of light (OD_{660}) with a Gilford Spectrophotometer (model 260). An OD_{660} reading needs to be at least 0.025 to be considered significant; the number, in general, represents a cell density of the order of 10^7 cells/ml. Therefore, significant growth above our baseline of approximately 10^7 cells/ml can be determined by this method.

Ester-linked PLFA analysis was used to identify groups of organisms from 50 bioreactor samples from the bioreactors in which growth was observed because these essential structural components of membranes are specific for different groups of microorganisms and also change as microorganisms adapt to changing environmental conditions. The proportion of living organisms, the nutritional status of the microbial community, and measures of aerobic versus anaerobic, and aerobic versus fermentative bacterial respiration [5] can all be determined by examining the components of the PLFA extracted from microbial communities.

Table I. Compositions of J-13 water, target maximum values from 100× concentrations of each component[1] and simulated steady-state aqueous compositions[2] used to design the target medium compositions (actual medium compositions are continuously monitored during the course of the experiments).

	J-13 [2]	Target[1] 100× J-13 values	Simulated[2] steady-state 100× J-13 +tuff	Target Med. 1	Target Med. 21	Target Med. 23	Target Med. 21B	Target Med. 23B	Target Med. 37A	Target Med. 37B
pH	~8.0		9.8	6.4	12	12	9.9	9.9	4.0	6.9
Na	4.58E+01	4.58E+03	4.57E+03	3.0E+02	7.0E+03	7.0E+03	1.0E+02	1.0E+02		2.0E+00
Si	2.85E+01	2.85E+03	9.91 E-01	2.0E+02	4.0E+03	4.0E+03	2.0E+02	2.0E+02		2.0E+02
Ca	1.30E+01	1.30E+03	8.67E+00	1.0E+01	5.0E+01	5.0E+01	1.0E+03	1.0E+03		4.0E+02
K	5.00E+00	5.00E+02	4.97E+03	8.0E+02	8.0E+02	8.0E+02	8.0E+02	8.0E+02		8.0E+02
Mg	2.01E+00	2.01E+02	2.88E+00	5.0E+01	5.0E+01	5.0E+01	3.0E+02	3.0E+02		1.5E+02
F	2.18E+00	2.18E+02	2.17E-01	7.0E-01	1.0E-01	1.0E-01	1.0E-01	1.0E-01		1.0E-01
Cl	7.10E+00	7.10E+02	7.13E-01	2.0E+00	1.0E+01	1.0E+01	3.0E+03	3.0E+03		4.5E+02
Li	4.80E-02	4.80E+00	4.79E+00	2.0E-02	2.0E-02	2.0E-02	2.0E-02	2.0E-02		2.0E-02
B	1.34E-01	1.34E+01	1.34E+01	2.0E-01	5.0E+00	5.0E+00	3.0E-01	3.0E-01		2.0E-01
Al	2.00 E-02	2.00 E+00	1.8E-02	5.0E-01	1.0E+00	1.0E+00	5.0E-01	5.0E-01		5.0E-01
Sr	4.00 E-02	4.00 E+00	n.e.	1.0E-01	5.0E-02	5.0E-02	1.0E-01	1.0E-01		1.0E-01
Fe[3]	~3.0E-02	~3.0E+00	5.5E-08	1.0E-01	1.0E-01	1.0E-01	1.0E-01	1.0E-01		1.0E-01
SO_4	1.84E+01	1.84E+03	1.8E+01	1.0E+02	2.8E+01	2.8E+01	3.6+01	3.6+01		1.5E+01
NO_3	8.80E+00	8.80E+02	8.76E-01	1.3E+01	5.0E+00	5.0E+00	1.3E+01	1.3E+01	1E+03	1E+01
PO_4 (max[4])	1.00E+01	1.00E+03	1.4E-04	3.5E+03	7E+00	7E+00	7E+00	7E+00		4.0E+03
HCO_3	1.29E+02	1.29E+02	2.7E-+00							
glucose	0.0	0.0	0.0	2E+03	0.0	2E+01	0.0	2E+01	2E+03	2E+03
duration[5] 21C exp.	n.a.	n.a.	n.a.	404	368	311	n.a	211	21	50
duration[5] 50C exp.	n.a	n.a	n.a.	298	207	207	n.a	207	13	63

[1] not a realistic aqueous solution; used for maximum values of individual elements in formulations
[2] simulated hypothetical 100 x J-13 water equilibrated with tuff using EQ3/6 aqueous geochemistry code [6,7]
[3] Fe has not been added to the medium formulations. It is expected to be available at saturation in most aqueous solutions in the drift environment because of the presence of waste packages.
[4] calculated from detection limit of J-13 analysis
[5] experimental duration (in days) as of October 30, 1998
n.a. not applicable; n.e. not evaluated

Biweekly bioreactor samples were filtered and analyzed by three methods. Using inductively coupled plasma atomic emission spectrometry (ICP-AES), the following elements are quantified: Al, Ca, Fe, K, Mg, Mn, Na, P, Si, Sr, S, B, Ba, Br, and Li. Ion chromotography is used to quantify the following aqueous species: F, Cl^-, NO^{2-}, NO^{3-}, PO_4^{3-}, SO_4^{2-}, acetate, formate, and propionate. The solution is also analyzed for total carbon (TC), total inorganic carbon (TIC), and total organic carbon (TOC). Scanning-electron-microscope (SEM) and x-ray diffraction analyses of the reacted tuff will be conducted at the completion of the experiments.

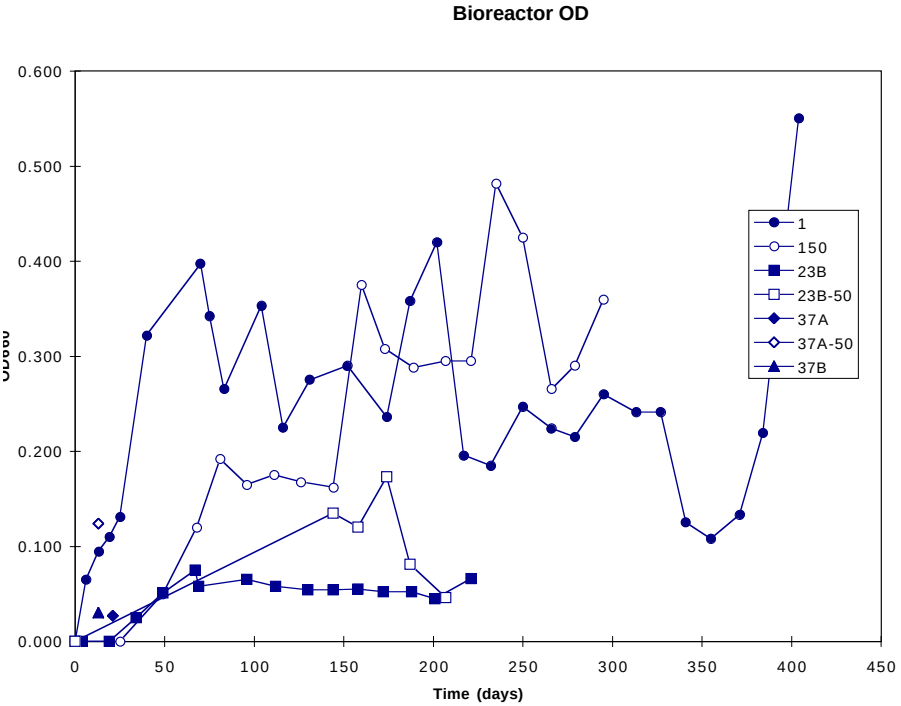
Many of the bioreactors are expected to show no growth. We ensure the continuing sterility of our system by periodically culturing the medium from each reservoir. Chemical analyses of the media are monitored over time as is the effluent from the bioreactors. Simulations of abiotic chemical reactions between tuff and media were conducted to establish an approximate expectation of the aqueous chemistry of the system in the absence of microbes. Both the EQ3/6 [6, 7], which models an aqueous system as it approaches equilibrium, and a coupled chemical–hydrologic code were used to examine the results of these experiments. Only the EQ3/6 results are discussed in this paper. To verify model results, sterile control experiments will be conducted for those bioreactors that show growth.

A sterile-water test was performed to examine the density of indigenous bacteria on the rock. Fifty grams of crushed rock (aseptically prepared, size between 1.7 mm and 2.8 mm) and 200 ml of

sterile nanopure water were contained in a sterile 500-ml flask. The flask was shaken vigorously by hand for several minutes and left to stand in a laminar-flow hood (sterile). Each time before a water sample was taken, the flask was shaken. Water samples were diluted and plated (0.1 ml) on duplicate R2A agar plates. The plates were incubated at 30°C. For up to 9 days of the flask incubation, no bacterial colonies were formed on the plates with a 100× dilution of the water sample. However, at day 12, we found that 992 and 1124 colonies were on the duplicate plates for the 100× diluted water, and 9 and 15 colonies were observed on the duplicate plates with the 10,000× dilution. The cell density was calculated to be $1.1 \pm 0.2 \times 10^6$ cells/ml water in flask or $2.3 \pm 0.5 \times 10^4$ cells/g crushed rock. We therefore defined "growth" as populations that increase significantly above that baseline population of approximately 10^6 cells/ml.

RESULTS

Growth has been observed in 21°C and 50°C bioreactors that were fed with media containing glucose at near-neutral pH (media 1 and 23B) (Fig. 1). The greatest cell densities were observed in reactors fed with the high glucose Medium 1. The cell densities in both bioreactors appeared to be oscillating, which is probably caused by interactions between different species of bacteria in the undefined, mixed bacterial cultures. Oscillatory phenomena in bioreactor variables (such as cell density and nutrient concentrations) are not uncommon for mixed bacterial population [8]. We note the possibility of oscillations in the community that might not be detected by PLFA assays (e.g., two species in the same genus [such as Bacillus] or two species that belong to two different but closely related geni). Growth in the Bioreactor 1-21C was relatively quick, requiring just 4 days; growth was observed in the Bioreactor 1-50C after 49 days. The lowest, but most consistent, cell density was observed in the Bioreactor 23B-21C. Growth in the Bioreactor 23B-50C was observed only recently. The growth had not been noticeable until four months after the experiment was initiated. Growth greater than that of Bioreactor 23B-21C was recorded at 144 days.



*Significant growth had already occurred in Bioreactor 23B-50C at 144 days (see text).

Figure 1. OD_{660} of bioreactors exhibiting growth.

Biomass was also measured in picomole of PLFA per mL of sample extracted and can be converted to cell numbers by assuming 1 pmol of PLFA to be equivalent to 2.5×10^4 cells. OD and PLFA pmol mL^{-1} correlated significantly across all four bioreactors. Bioreactor 1-50C resulted in the greatest biomass (over time), followed by Bioreactor 1-21C, then 23B-50C, and finally 23B-21C. Greater variations (magnitudes) in biomass were seen from sample to sample with the PLFA assay, which may be attributed to a greater sensitivity of the biomass measure or to the smaller sample size. Microbial biomass of Bioreactor 1-21C did not vary significantly; that of Bioreactor 23B decreased over the time-course study. The microbial biomass in Bioreactor 1-50C increased slightly over the

time course of the experiments. The microbial biomass measured in Bioreactor 23B-50C was comparable to that measured in reactor 23B.

Although the metabolism of glucose is probably responsible for the change in pH exhibited by Bioreactor 1-21C, only selected inorganic chemical results can be discussed in this short paper. Bioreactor 1-21C (Fig. 2) exhibited a significant drop in pH, from the pH of the medium (6.2) to as low as 3.9, and reached a fairly stable value after 100 days. F– and Cl– concentrations appear to have a fairly long-range (2–3 months) cyclic behavior; Fe concentration appears to increase, and that of Ca appears to decrease slightly. At 50°C, Bioreactor 1-50C (Fig. 3), which has the same medium and tuff and initial microbial community, exhibited a fluctuation in pH from 6.2 to 4.8 and back, but never as low as did Bioreactor 1-21C. In contrast to observations in Bioreactor 1-21C, the concentration of Ca and Cl– in Bioreactor 1-50C appeared to increase, markedly so in the case of Cl–. Neither F– nor Cl– exhibited the periodic behavior apparent in Bioreactor 1-21C. It is the concentration of Fe that showed some appearance of periodic change. In both reactors, the concentrations of PO_4^{-3}, SO_4^{-2}, K, Na, Mg, and Si remain fairly constant. Al, Sr, Li, B, Ba were below or near the detection limit.

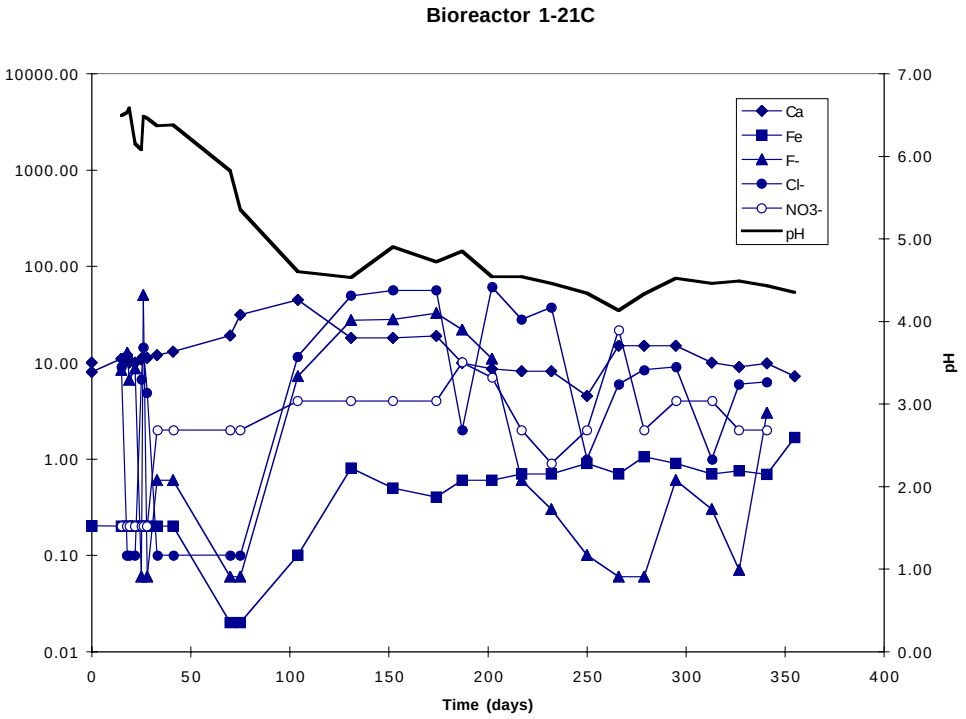


Figure 2. Selected chemical results from Bioreactor 1-21C illustrating a striking decrease in pH, an increase in the Fe, and possibly a periodic variation in Cl– and F– concentrations.

Nutrient usage was examined by comparing molar ratios of SO_4/S, PO_4/P, and $NO_3/(NO_2+NO_3)$ concentrations over time. Figure 4 compares the SO_4/S signatures of longer-duration bioreactors, with and without growth. Bioreactor 23B-21C exhibited a signature distinctly different from those of the other bioreactors that show growth and all of the nongrowth bioreactors in which SO_4 concentration is significantly reduced compared to total S concentration. Note also the cluster of early stage Bioreactor 1-21C values, which are also in that range.

Results of the PLFA assay, analyzed using factor analysis indicate that distinct biomasses and community compositions exist in each of the four bioreactors exhibiting growth. Similarities in PLFA characteristics exist across medium chemistries at the same temperature, and some evolution of the communities was observed. Comparison of PLFA community profiles along with additional variables such as pH and biomass yielded similar results.

Bioreactor 1-21C samples exhibited strong similarities among themselves in the presence of cyclopropyl PLFA (cy17:0 and cy19:0), which are abundant in a number of Gram-negative bacteria (e.g., *Alcaligenes, Enterobacter, Nitrobacter, Pseudomonas, Vibrio*). The formation of cy17:0 and cy19:0 may also be induced under conditions of nutrient deprivation. A small increase in the relative percentage (mole%) of polyunsaturated PLFA (after day 150) may be due to the initiation of grazing of the Gram-negative bacteria by micro-eukaryotes. For Bioreactor 1-21C, PLFA abundance (biomass) correlated inversely to pH, which is notable because the pH of the medium is considered ideal for most bacterial growth. Actinomycetes, which as a group tend to prefer lower pH, may be a

significant component of these reactor communities. Two samples taken from two early time points (days 28 and 70) showed a similarity to samples recovered from Bioreactor 23B-21C.

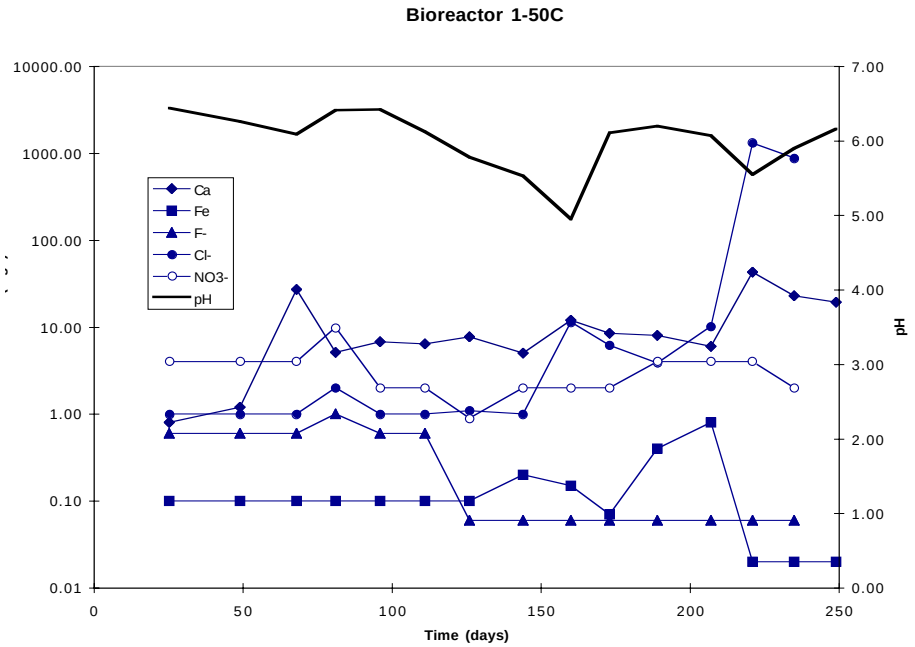


Figure 3. Selected chemical results from Bioreactor 1-50C, illustrating fluctuations in the pH, an increase in Cl concentration after 200 days, and a decrease in Fe concentration

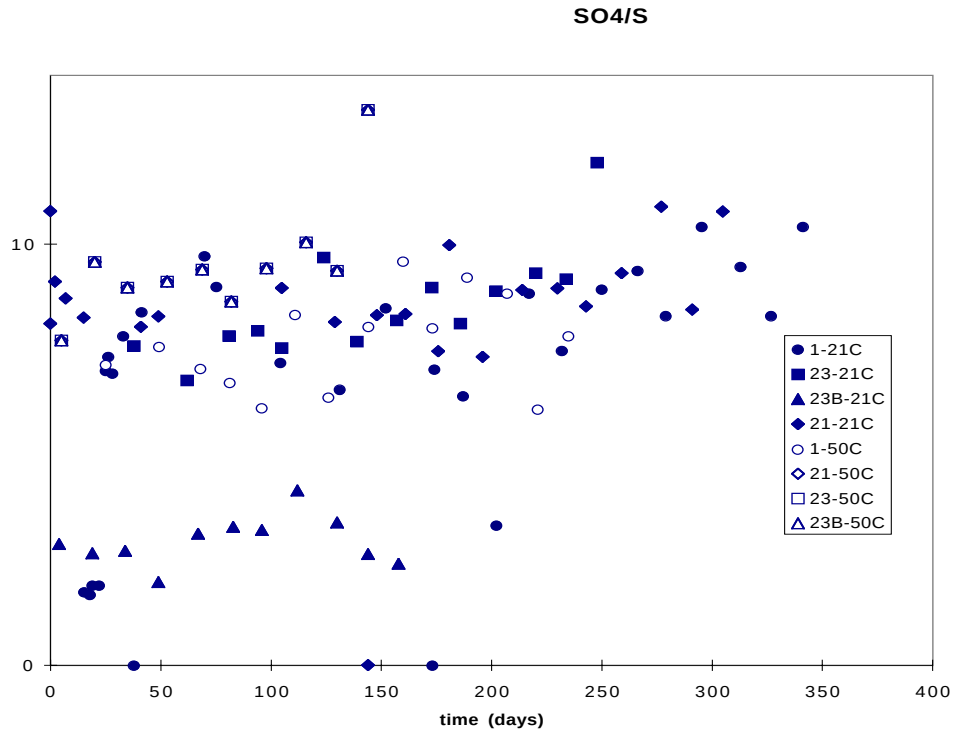


Figure 4. Mole ratios of SO_4 to total sulfur concentration plotted through time illustrate the clustering of most bioreactors between 5 and 10. Bioreactor 23B-21C and a few early points from 1-21C display a distinctly lower SO_4/S signature, which correlates with the PLFA indication of the presence of sulfur-reducing bacteria (SRB).

Bioreactor 23B-21C samples exhibited greater concentrations than did other bioreactors of the monounsaturate 18:1w7c, which is indicative of several methanotrophic bacteria (e.g., *Methylobacterium*, *Methylocystis*, and *Hyphomicrobium*), but contributions from other Gram-negative bacteria

such as *Pseudomonas* are highly possible and likely. An increase in terminally branched, saturated (Gram-positive bacteria) and branched monounsaturated (anteiso rather than iso methyl branched) PLFA in the middle of the time course studied appear to be from Arthrobacter-like organism(s). This change in the PLFA signature probably represents a shift in the reactor biota due to an environmental stimuli or to natural succession. This interpretation is supported by the cyclopropyl/monounsaturated precursor ratio, an indication of short-term stress, which began to increase to a substantial level on day 144, coincident with the end of the Gram-positive plume.

Bioreactor 1-50C exhibited the greatest OD and PLFA biomass (which are themselves correlated) and the largest percentage of terminally branched saturated PLFA (typical of Gram-positive bacteria). The iso configuration of terminally branched, saturated PLFA was more abundant than was the anteiso, which indicates the likely presence of the *Bacillus* or *Micrococcus* genera. Although the community composition varied little over the course of study, a decrease in Gram-negative bacterial biomarkers was also observed between days 68 and 207. Bioreactor 23B-50C, although lower in biomass, exhibited a microbial community composition more similar to those identified in Bioreactor 1-50C samples than in those of Bioreactor 23B-21C.

DISCUSSION

EQ3/6 [6, 7] simulations representing the reaction of Medium 1 with tuff at 25°C and 50°C under normal atmospheric conditions as it approaches a steady state, were conducted in various modes with reaction kinetics [9] or best judgment based on these values. The results for the various modes, although different in important aspects that cannot be reported in this short paper, did not differ with respect to the salient points of this discussion. In simulated reactions at both 25°C and at 50°C, pH increased to approximately 8 at steady state. Concurrent changes in chemistry (related to the dissolution of the tuff minerals and the precipitation of other minerals) included a drop in the concentration of phosphorus, fluoride, potassium, and magnesium in solution and an increase in carbonate and aluminum. For perspective on a flow-through system, we note that a stable chemical signature (that of the original medium) would result if the chemical reaction between the tuff and the medium or mediated by microorganisms were insignificant compared to the incoming concentration, if the flow rate of the reactor were fast relative to the rate of the chemical reactions, or if the volume of medium were much higher than the reactive surface of the tuff. The interaction between flow rate and reaction rate can be demonstrated with coupled hydrologic—chemical codes [10]. Although the chemistry in such simulations is reduced, the perspective that can be gained is useful.

The simulated approach to steady state of the abiotic reaction between the medium and the tuff is strikingly different from the trends observed in Bioreactors 1-21C and 1-50C. Bioreactor 23B-21C significantly modified the sulfur signature in a manner not imitated by Bioreactor 23B-50C. That signature, in both bioreactors 23B-21C and 1-21C, correlated with the PLFA results, indicating a predominance of SRB. This clearly demonstrates that microbially mediated chemistry contributes significantly to the chemistry of the solution, even at a ratio of 100 gm crushed tuff to 400 ml medium. That microbially mediated chemistry is significant but cannot be reproduced using our present abiotic chemical simulation tools.

For chemical modeling purposes, we restate the well-known idea that microorganisms adapt to and take advantage of their environment as follows: the microbial community is determined by the chemistry and the temperature of its environment. The chemically selected communities utilize the available resources, to modify the aqueous chemistry, by processes that are also fairly well defined Less well recognized is that this represents a feedback mechanism between microbially mediated chemistry and process-level abiotic aqueous chemistry that need not be species-specific. In the interests of developing such a model, the experiments were used to identify, in addition to microbial growth (which, as a function, yields cell density), three categories of microbially mediated chemical effects, each of which can be described by a different constitutive equation. The first effect is the unidirectional bacterial modification of the chemistry (i.e., pH in Bioreactor 1-21C or Cl^- in Bioreactor 1-50C). Parameters for this equation will include cell density and a reaction rate. The second are cyclical effects (e.g., F in Bioreactor 1-21C) that may be attributed to internal regulation (e.g., osmoregulation or internal pH regulation) or to interactions between different bacterial species, such as competition for nutrients between the species in a mixed population. As with the first effect, the equation will be parameterized by cell density and a rate, but will also be described by a periodic function. The third is the secondary impacts of the above direct microbial effects, such as the increased dissolution of solids as a result of reduced pH. Given the direct microbial modification, these effects will be simulated using the abiotic chemical models that are currently available.

SUMMARY AND CONCLUSIONS

From identical initial colonies and mineral constituents in flow-through bioreactor experiments, medium chemistry and temperature have determined the character of distinct microbial communities with characteristic PLFA signatures. These communities can significantly affect the resulting effluent chemistry. This demonstration shows that process-based models of microbially mediated aqueous chemistry do not necessarily depend on the identification or evaluation of each constituent species; they can work at the level of whole communities. The chemical data from the experiments have been obtained in a form that is easily reconciled with process-based chemical models: a measure of the microbial community at the appropriate scale (PLFA technique is impressive this respect); experiments long enough to exhibit any long-term periodic chemical fluctuations; and detailed chemical documentation and control of variables factors using flow-through reactors and well-characterized, carefully tailored media formulations and minerals.

The chemically/thermally determined microbial communities have distinct chemical impacts that can also be distinguished from the simulated abiotic aqueous chemical reactions between the media and the tuff. Three types of microbially mediated chemical effects (as well as microbial growth), each of which will require a different constitutive equation, are distinguished in this study: 1) unidirectional bacterial modification of the chemistry (i.e., pH), 2) secondary impact of direct microbial modifications (i.e., increased dissolution of solids as a result of reduced pH), and 3) cyclical effects that may be attributed to internal regulation (e.g., osmoregulation or internal pH regulation) or cyclical evolution of the microbial communities.

ACKNOWLEDGMENTS

Thanks to R. Pletcher, J. Miller, and P. Ernst for preparation of the tuff and to C. Aracne and M. Spragge for its collection from the ESF. This work was performed under the auspices of the U.S. Department of Energy by Lawrence Livermore National Laboratory under Contract W-7405-ENG-48, and is supported by the Yucca Mountain Site Characterization Project, LLNL.

REFERENCES

1. T.L. Kieft, W.P Kovacik, D.B. Ringelberg, D.C. White, D.L. Haldeman, P.S. Amy, and L.E. Hersman. *App. and Environ. Microbiol.* **63** p. 3,128 (1997).

2. A. Matin, E.A. Auger, P.H. Blum, and J.E. Schultz, *Annu. Rev. Microbiol.* **43,** p. 293–316 (1989).

3. J.E. Harrer, J.F. Carley, W.F. Isherwood, and E. Raber, UCID-21867, Lawrence Livermore National Laboratory, Livermore, CA (1990).

4. American Type Culture Collection (ATCC). *Catalogue of bacteria and bacteriophages*. 18th ed. ATCC, Rockville, MD (1992).

5. D.B. Hedrick and D.C. White, *J. Microbiol. Meth.* **5**: 243 (1986).

6. T.J. Wolery. UCRL-MA-110662, Lawrence Livermore National Laboratory, Livermore, CA. (1992).

7. T.J. Wolery and S.A. Daveler. UCRL-MA-110662, Lawrence Livermore National Laboratory, Livermore, CA (1992).

8. W.E. Glassley, W.L. Bourcier, and J.W. Johnson, UCRL-ID-124846, Lawrence Livermore National Laboratory, Livermore, CA (1998).

9. J.W. Johnson, K.G. Knauss, W.E. Glassley, and L.D. DeLoach, *Journal of Hydrology* (in press).

10. J.E. Bailey and D.F. Ollis. *Biochemical Engineering Fundamentals*, McGraw-Hill, New York (1986), Chapter 13.